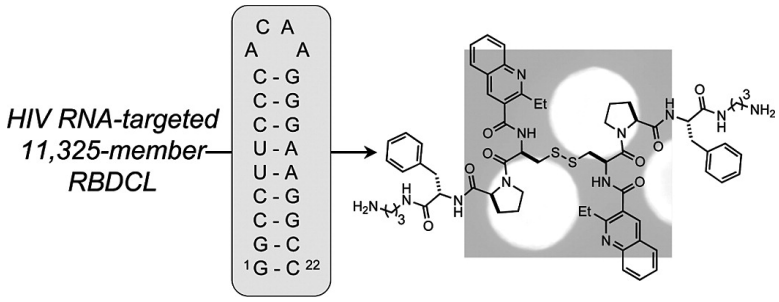
Communication

Identification of a Selective Small-Molecule Ligand for HIV-1 Frameshift-Inducing Stem-Loop RNA from an 11,325 Member Resin Bound Dynamic Combinatorial Library

Brian R. McNaughton, Peter C. Gareiss, and Benjamin L. Miller

J. Am. Chem. Soc., **2007**, 129 (37), 11306-11307• DOI: 10.1021/ja072114h • Publication Date (Web): 28 August 2007



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 9 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 08/28/2007

Identification of a Selective Small-Molecule Ligand for HIV-1 Frameshift-Inducing Stem-Loop RNA from an 11,325 Member Resin Bound Dynamic Combinatorial Library

Brian R. McNaughton,[†,§] Peter C. Gareiss,[¶,§] and Benjamin L. Miller*[,‡,¶,§]

Departments of Dermatology, Biochemistry and Biophysics, and Chemistry, and the Center for Future Health, University of Rochester, Rochester, New York 14642

Received March 26, 2007; E-mail: benjamin_miller@futurehealth.rochester.edu

It has been estimated that 39.5 million people are infected with the Human Immunodeficiency Virus (HIV) worldwide, 4.3 million of these becoming infected in 2006 alone.[1] Expression and proteolysis of the polyprotein Pol is required for the production of three proteins vital to viral proliferation (HIV-integrase, -protease, and -reverse transcriptase). Pol is produced *only* as a Gag-Pol fusion protein, which is translated 5−10% with respect to Gag (depending on the technique used for measurement), via a tightly regulated −1 nucleotide ribosomal frameshift (Figure 1).[2−4] Two principal factors responsible for this frameshift are (i) a UUUUUUA "slippery sequence" where the frameshift occurs, and (ii) a highly conserved downstream stem-loop which has been shown to play a vital role in frameshifting.[5] Precise control of frameshifting is essential to viral proliferation, as small changes in Gag-Pol expression levels drastically inhibit virus production.[6−8]

Small molecules able to selectively bind the HIV-1 frameshift regulatory sequence should have utility as inhibitors of viral replication, as well as helping to address the widely recognized goal of identifying new RNA-selective compounds.[9] As a first step in the development of such compounds, we have employed a recently reported new method for constructing and analyzing dynamic combinatorial libraries (DCLs) termed resin-bound dynamic combinatorial chemistry (RBDCC).[10] RBDCC circumvents constraints on library size, imposed by the need for post-screen identification of selected (active) compounds from the library mixture, which can diminish the utility of solution-phase DCLs. In the RBDCC approach, library building blocks are covalently attached to a solid support and allowed to equilibrate in the presence of solution-phase building blocks and labeled target. Simple cleavage and analysis of resin beads bearing the labeled target identifies building blocks present in selected library members. Herein, we describe the application of RBDCC to the generation and screening of a DCL with a theoretical size of 11,325 members, the largest prepared to date, targeting the HIV-1 frameshift regulatory stem-loop RNA (nucleotides 1−22, boxed sequence, Figure 1).

The library design expands on our previously reported DNA-binding analogues of the bis-heterocyclic octadepsipeptide family of nucleotide-binding natural products.[10] We prepared 150 building blocks, which when allowed to equilibrate by disulfide exchange generate a theoretical diversity of 11,325 unique library members. In addition to structural diversity provided by the incorporation of a range of amino acids and carboxylic acids in the library, variation in the position of the cysteine residue provided further library complexity. Solution-phase building blocks were prepared on Wang resin by split-pool synthesis, cleaved, and used as a heterogeneous mixture. Resin-bound building blocks (cysteine thiol-*S*-^{t}Bu disulfide protected) were prepared on three different sizes of TentaGel (50 building blocks per resin size) to encode the position of the cysteine residue (Scheme 1). Resin loading across the three different size beads was normalized at 0.86 nmol/bead by blocking medium and large size resin with appropriate amounts of methoxyacetic acid. A previously described photolabile linker[11] was incorporated to allow for cleavage of molecules after analysis.

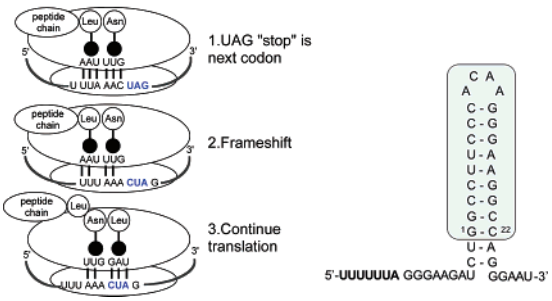
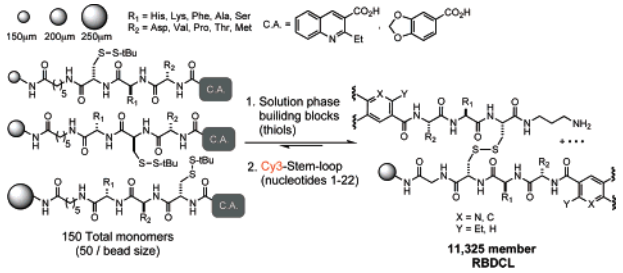


Figure 1. Left: Ribosomal frameshifting schematic. Right: HIV-1 slippery sequence (bold) and downstream regulatory stem-loop (boxed).

Scheme 1. HIV-1 Targeted RBDCL



First, control experiments were conducted to ensure that monomeric resin-bound building blocks do not bind the RNA under the screening conditions used. Next, resin-bound DCLs were prepared by equilibrating a heterogeneous mixture of solution-phase building blocks (30 μM based on average molecular weight) with resin-bound building blocks (387 μM) and Cy-3-labeled stem-loop RNA (1 μM) in phosphate buffer (PBS, pH 7.4). Libraries were equilibrated in quadruplet for 72 h, a period of time shown by HPLC to be sufficient for equilibrium to be reached. The resin was then drained, washed, plated with 2 mL buffer in a petri dish, and analyzed by fluorescence microscopy. The three beads with highest fluorescence were removed via syringe, washed, and subjected to photolytic cleavage (365 nm) for 24 h. The resulting solution was analyzed by mass spectrometry to identify unreacted thiol-*S*-^{t}Bu monomers, which as the highest population species on the resin were the most easily detected. Masses corresponding to building blocks **1**, **2**, and **3** were observed (Figure 2). Importantly, all three of these building blocks were also selected in replicate experiments. Therefore, it was concluded that only dimers produced from a combination of these building blocks (nine possible compounds)

‡ Department of Dermatology.
¶ Department of Biochemistry and Biophysics.
† Department of Chemistry.
§ Center for Future Health.

10.1021/ja072114h CCC: $37.00

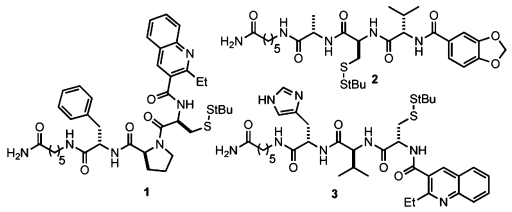


Figure 2. Library building blocks selected by RBDCC.

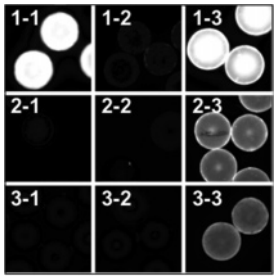


Figure 3. Determination of the highest-affinity ligands (numbering scheme x−y: x = resin-bound component, y = solution-phase component).

were possible RNA-binding ligands. Additionally, screening this library with a different RNA sequence reproducibly yielded a completely different set of hits (data not shown).

Identification of these three building blocks then paved the way for us to determine the highest affinity ligand that can be made from all possible combinations of the selected monomers. Compounds **1**, **2**, and **3** were individually synthesized on TentaGel (0.86 nmol/bead), equilibrated in separate vessels containing only solution phase **1**, **2**, or **3** prepared as the thiol, and 500 nM Cy-3-labeled RNA (half the concentration used in the initial screen). After 72 h, the resin was washed, and the nine experiments were analyzed by fluorescence microscopy (Figure 3).

As indicated by bead fluorescence, dimers **1-1** and **1-3** had the highest affinity for the RNA stem-loop. Beads corresponding to **1-3** exhibited high fluorescence intensity, while those corresponding to **3-1** did not. This highlights an important feature of RBDCC: solution-phase and resin-bound components are in competition for target binding. For example, if a dimer formed in the solution phase is of higher affinity than any of the homo- or heterodimers formed on resin, the labeled target will bind preferentially to the solution-phase dimer. This will result in the labeled target being washed away at the end of the screen, leading to low bead fluorescence. In contrast, if the highest affinity dimer can be formed on resin, binding will occur on this phase and one will see high bead fluorescence at the end of the screen. On the basis of this analysis, the results shown in Figure 3 led us to expect that **1-1** would have an affinity higher than that of **1-3** for the RNA stem-loop.

To confirm this hypothesis, dissociation constants (K_d) were measured for dimers **1-1** and **1-3** by surface plasmon resonance (SPR) in PBST, pH 7.4.[12] Compound **1-1** was found to have an affinity of 4.1 ± 2.4 μM to the HIV-1 frameshift regulatory RNA sequence (sequence I, Figure 4), while the affinity of **1-3** was determined to be >90 μM. Specificity was assessed by measuring the affinity of **1-1** to four other oligonucleotides: a DNA sequence (II) homologous to I, an alternate RNA stem-loop (III),[13] an RNA stem-loop with an altered loop sequence (IV), and a shortened version of I (V; Supporting Information). Gratifyingly, **1-1** showed no measurable affinity for sequences II, III, and V and only marginal (>90 μM) affinity for sequence IV. These results confirm that, of the compounds selected, **1-1** has the highest affinity for the HIV-1 frameshifting regulatory RNA sequence (I) and also indicate that

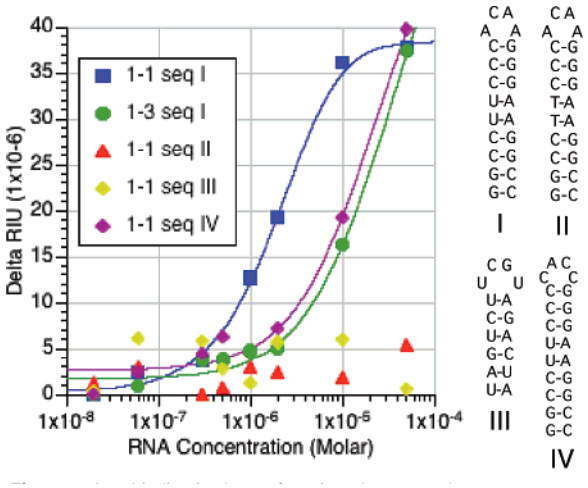


Figure 4. SPR binding isotherms for selected compounds.

the recognition process is exceptionally sequence-selective. As further evidence of selectivity, we also verified that bead-bound **1-1** is able to bind Cy-3-labeled RNA sequence I in the presence of a large excess of tRNA.

In conclusion, we have used the RBDCC method to generate and screen a DCL with a theoretical diversity of 11,325, the largest prepared to date. While this library was small enough to exclude mass overlap of library building blocks, much larger RBDCLs with mass overlap could be deconvoluted using resin-bound tagging schemes. From this screen, we identified a selective and high-affinity ligand for the HIV-1 frameshift-inducing stem-loop, a vital RNA regulator of the HIV-1 life cycle. Efforts are underway to further enhance the affinity of **1-1** for stem-loop RNA sequence I via exploration of structure−activity relationships and to assess the ability of related compounds to inhibit viral proliferation.

Acknowledgment. B.R.M. and P.C.G. were supported by the NIH (T32AR007472). We thank Dr. Alice Bergmann (University of Buffalo) for mass spectroscopy data (NSF award CHE0091977), and Charles Mace (University of Rochester) and Thomas Ryan (Reichert Instruments) for assistance with SPR.

Supporting Information Available: Detailed experimental procedures and spectral analysis of compounds **1-1** and **1-3**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) See: http://www.unaids.org/en/HIV_data/2006GlobalReport/default.asp.
(2) Park, J.; Morrow, C. D. *J. Virol.* **1991**, *65*, 5111−5117.
(3) Jacks, T.; Power, M. D.; Masiarz, F. R.; Luciw, P. A.; Barr, P. J.; Varmus, H. E. *Nature* **1988**, *331*, 280−283.
(4) Parkin, N. T.; Chamorro, M.; Varmus, H. E. *J. Virol.* **1992**, *66*, 5147−5151.
(5) Telenti, A.; Martinez, R.; Munoz, M.; Bleiber, G.; Greub, G.; Sanglard, D.; Peters, S. *J. Virol.* **2002**, *76*, 7868−7873.
(6) Karacostas, V.; Wolffe, E. J.; Nagashima, K.; Gonda, M. A.; Moss, B. *Virology* **1993**, *193*, 661−671.
(7) Hung, M.; Patel, P.; Davis, S.; Green, S. R. *J. Virol.* **1998**, *72*, 4819−4824.
(8) Shehy-Xhilaga, M.; Crowe, S. M.; Mak, J. *J. Virol.* **2001**, *75*, 1834−1841.
(9) (a) Thomas, J. R.; Liu, X.; Hergenrother, P. J. *J. Am. Chem. Soc.* **2005**, *127*, 12434−12435. (b) Tor, Y. *ChemBioChem* **2003**, *4*, 998−1007. (c) Sucheck, S. J.; Wong, C.-H. *Curr. Opin. Chem. Biol.* **2000**, *4*, 678−686.
(10) McNaughton, B. R.; Miller, B. L. *Org. Lett.* **2006**, *8*, 1803−1806.
(11) Brown, B. B.; Wagner, D. S.; Geysen, H. M. *Mol. Div.* **1995**, *1*, 4−12.
(12) (a) Whitney, A. M.; Ladame, S.; Balasubramanian, S. *Angew. Chem., Int. Ed.* **2004**, *43*, 1143−1146. (b) Lacy, E. R.; Le, N. M.; Price, C. A.; Wilson, W. D. *J. Am. Chem. Soc.* **2002**, *124*, 2153−2163. (c) Carrasco, C.; Facompre, M.; Chisholm, J. D.; Van Vranken, D. L.; Wilson, W. D.; Bailly, C. *Nucleic Acids Res.* **2002**, *30*, 1774−1781.
(13) Karan, C.; Miller, B. L. *J. Am. Chem. Soc.* **2001**, *123*, 7455−7456.

JA072114H